Review

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Challenges in Metabolite Biomarkers as Avenues of Diagnosis and Prognosis of Cancer

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Given the evolutionary nature of tumor complexities and heterogeneity, the early diagnosis of cancer encounters various challenges. Complexities at the level of metabolite reprogramming are compelling in the background of invasiveness, metastasis, drug- and radiation-induced metabolic alterations, immunotherapy-influenced changes, and pro-tumor niche including microbiome. Therefore, it is crucial to examine both current and future obstacles associated with early cancer detection specifically in the context of tumor metabolite biomarkers at preclinical and clinical levels. In conclusion, the significance of tumor metabolite biomarkers must be aligned with a comprehensive approach to achbieve diagnosis and prognosis of cancer patients by securing solutions to formidable challenges.

Key Words Biomarkers, Neoplasms, Metabolomics, Diagnosis, Metabolic reprogramming

INTRODUCTION

Cancer has become a serious threat to the human race and the numbers have risen radically in the past few years. According to World Health Organization, over 10 million deaths were caused by cancer in the year 2020 globally and it has been estimated to increase by 49.7% by 2040 [1,2]. The complex nature of tumors is characterized by many distinctive hallmarks, significantly impacting their early detection and treatment. Among these features, the lack of reliable and affordable early detection tools and drug resistance stands out as a critical challenge, complicating the effectiveness of therapies and the management of cancer patients. Tumors are also adept at evading the body's immune response and adapting to changing conditions, contributing to their survival and progression. This multifaceted behavior not only hinders the development of early detection and prognosis of cancer patients but also necessitates a concerted efforts by biochemists, molecular biologists and clinicians to improve patient outcomes. The intricate and dynamically evolved characteristics of tumors present a significant obstacle to the integration of early diagnosis strategies for effective cancer patient management [3-5].

Indeed, early diagnosis of cancer undoubtedly contributes to improved patient survival rates. Numerous endeavors have been undertaken to underscore the difficulties and current status of technologies that may facilitate the imperative for early cancer diagnosis [6-8]. For the need for cancer early detection, substantial endeavors have been made by harnessing state-of-the-art technologies encompassing genomics, proteomics, epigenomics, and metabolomics [7-11].

These advanced methodologies are instrumental in the analysis of intricate biological specimens including serum, saliva, urine, nails, tears, and sweat. Nonetheless, complexities of biological samples are added with intra- and inter-tumor heterogeneity and metabolic reprogramming crucial tumor hallmarks [5-11]. These well-known attributes of tumors contribute to various challenges to achieve affordable and accessible with better sensitivity and specificity on tumor metabolite biomarkers for diagnosis and prognosis of cancer patients. This paper addresses the key challenges in tumor metabolite biomarkers at preclinical and clinical levels with future perspectives on tools and technologies that pave the pathways for better diagnosis and prognosis of cancer patients.

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METABOLIC REPROGRAMMING IN CANCER

Cancer cells undergo metabolic changes to support their uncontrolled growth, meet their energy requirements, and compromise their microenvironment. Aerobic glycolysis, lipid reprogramming, and amino acid metabolism are some of the key alterations that occur in cancer cells for their survival [12-18].

Tumors frequently induce hypoxia, reducing oxygen levels as a consequence of heightened energy consumption. In aerobic glycolysis, a metabolic shift occurs where glucose is converted to pyruvate, followed by the formation of lactate, even in the presence of oxygen [19-22]. This metabolic adaptation is triggered by the activation of hypoxia-inducible factor 1-alpha under hypoxic or normoxic conditions, as well as through the inactivation of tumor suppressor genes, activation of oncogenes, perturbed signaling of growth factors, and interactions with components of the tumor microenvironment [20-25].

The findings of tumor heterogeneity paved the way for the revelation of the reverse Warburg effect, where the transfer of metabolites like lactate from cancer cells undergoing aerobic glycolysis to the adjacent cancer cell occurs. This supports the neighbor cancer cells for ATP production, growth, and proliferation via oxidative phosphorylation [10-20]. Although not all cancer types exhibit the Warburg effect, other pathways like the pentose phosphate pathway, and tricarboxylic acid (TCA) cycle are also involved in metabolic alterations and contribute to cancer cell survival [25-29].

Lipid reprogramming is a rising hallmark of cancer. The three main lipid classes, fatty acid (FAs), phospholipids, and cholesterol play an important role in cell membranes, cell integrity, signaling, and lipotoxicity, and are dysregulated in tumors. The source of FAs in the tumor is mainly through de novo synthesis. Recent studies supported that tumors also take FAs from the tumor microenvironment stressing their importance in cancer viability [8-12]. Lipogenesis, lipolysis, and lipid storage are seen upregulated in many cancer cells to fulfill their basic energy needs and requirements. The intermediates of lipid metabolism can escalate the activation of oncogeneic pathways in cancer resulting in tumor progression and metastasis. Element-like transcription factor, signaling pathways include NF-κB, phosphatidylinositol-3 kinases/Ak strain transforming, Salvador-Warts-Hippo, mitogen-activated protein kinase, Wingless-related integration site/β-catenin, adenosine 5′-monophosphate-activated protein kinase, Notch, and STAT3, and non-coding RNA are found to contribute to lipid metabolic reprogramming in the tumor [15-20].

The main functions of amino acids are energy regulation, homeostatic maintenance, biosynthetic support, and redox balance which has drawn interest in amino acid metabolism in cancer [8-12]. For instance, glutamine, a particular type of amino acid acts as a replenishing intermediate of the TCA cycle, involved in synthesizing lipids, proteins, and nucleic acids. The catabolism of amino acid produces metabolic intermediates that affect cancer growth and survival and also connect other metabolic processes resulting in cancer cell existence. In addition, these intermediates also act in the regulation of epigenetics changes and post-translational modification such as histone modification, DNA methylation, chromatin remodeling, and noncoding RNA-induced modification leading to tumorigenesis [25-29].

Metabolic alterations in the contexts of pathways such as glutamine, polyamine, and nucleotide metabolism play a crucial role in the broader landscape of abnormal metabolism observed in various diseases [12-15]. These metabolic disruptions are intricately linked to well-documented abnormalities including mitochondrial dysfunction, glycolysis imbalances, disruptions in the TCA cycle, and alterations in amino acid metabolism. For instance, glugaminosys, a process involving the metabolism of specific amino acids, can be significantly altered in pathological conditions, leading to impaired cellular function and disease progression [16-20].

Similarly, polyamine metabolism, which is essential for cell growth and differentiation, often undergoes changes that contribute to abnormal cell proliferation and tumor development. Nucleotide metabolism, crucial for DNA and RNA synthesis, can also be disrupted, affecting cellular replication and repair mechanisms [20-25]. These metabolic disturbances are frequently accompanied by mitochondrial dysfunction, where the energy production and oxidative phosphorylation processes are compromised. Additionally, altered glycolysis and TCA cycle activities can lead to an accumulation of metabolic intermediates that further exacerbate disease states [25-29]. Overall, understanding these interconnected metabolic path-

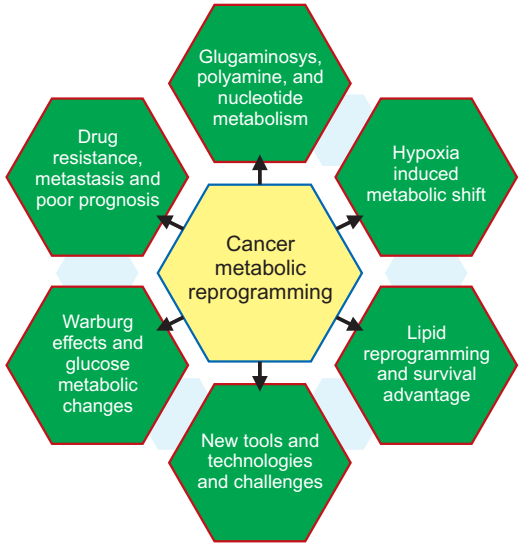

Figure 1. Metabolic reprogramming in cancer and their various facets that influence the outcome of metabolite biomarkers as diagnosis avenues.

ways and their abnormalities is vital for developing targeted therapeutic strategies and improving treatment outcomes for various metabolic disorders and cancers.

In summary, metabolites are biochemical footprints that provide insights on various diseases and conditions. These can be in the form of carbohydrates, lipids, nucleosides, amino acids, enzymes, vitamins, alcohols, and organic acids (Fig. 1). These metabolic products can be useful as biomarkers, clinical diagnosis, prognosis, and disease classification. Thus, understanding or identifying these small metabolites or metabolic changes helps in providing clarity on disease pathophysiology and discovering therapeutic targets.

TOOLS AND TECHNIQUES BEHIND METABOLIC BIOMARKERS

For several decades, the field of "omics" biology has emerged as a highly diversified branch of science encompassing genomics, proteomics, and metabolomics, all of which bear the suffix "-omics" in their names [30-33].

Essentially, the field of omics enables the characterization and quantification of various large and small biomolecules, thereby contributing to the fundamental, preclinical, and clinical aspects of biological science. The term metabolomics was coined over two decades ago to parallel genomics, to comprehensively characterize small molecule metabolites at the cellular, tissue, and organism levels. Metabolomics serves as an advanced platform for the characterization of metabolites in cells, tissues, and bio-fluids, and it is widely employed in translational research, particularly in the detection of various human diseases, including tumors [34-39].

In the current landscape, in addition to genomics and proteomics analysis of metabolic changes, diverse analytical methodologies have emerged for performing metabolomics investigations, including liquid chromatography-high resolution mass spectrometry, nuclear magnetic resonance spectroscopy, Fourier-transform ion cyclotron resonance mass spectrometry, and indirect calorimetry [30-39]. Furthermore, metabolic imaging techniques like positron emission tomography, and MRI are also utilized to track down metabolic defects in body tissue. Besides the high end imaging and spectrometry technqiues, the development of 96-well-plate colorimetric assays for the specific set of metabolites such polyamines, amino acids and lipids are being encouraged in terms of cost effective and accessibility of the cancer patients [25-35]. However, challenges concerning sensitivity arise due to the intricate composition of biological samples, encompassing both targeted and untargeted metabolites, sought as potential biomarkers for the early detection of cancer. These challenges in metabolomics require concerted strategies for effective resolution [25-35]. A summay of tools and techniques for metabolite biomarkers in cancer patients is presented (Fig. 2).

To achieve metabolic biomarkers as diagnostic tools, a non-invasive approach by exploring biomarkers in urine, saliva, tears, nail clippings, and sweat could be explored in the current scenario of increasing burdens of cancer in developing and poor countries where issues of affordability and accessibility are major constraints. The potential use of biological fluids and materials such as urine, saliva, tears, nail clippings, and sweat could be highlighted as advantages in the forms of discard-to-detection approaches for cancer [40-50].

CHALLENGES IN METABOLIC BIOMARKER DISCOVERY

At the level of tumor heterogeneity, molecular noise represents a primary challenge, as it drives the complexity of this disease. Furthermore, this molecular noise is influenced by interactions between normal cells, cancer cells, and the microbiome of the human body, which adds a layer of complexity that can impact the efficacy of early cancer diagnosis strategies [5-12]. Inconsistency in laboratory results due to the selection of tests according to subject conditions, availability of various platform options for a particular test that leads to different outputs and cutoff values, and complex diagnostic results that contain multiple layers of information also affect the cancer diagnosis [10-12].

The second challenge is well discussed in this paper and

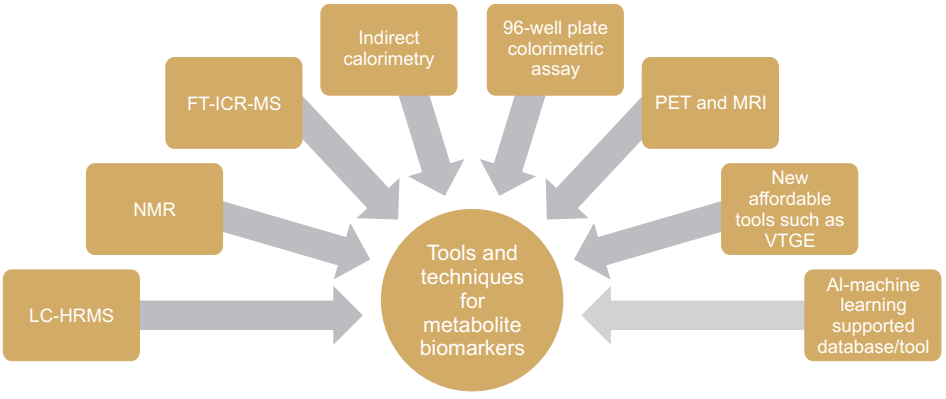

Figure 2. Various tools and techniques for the development of metabolite biomarkers in cancer patients. LC-HRMS, liquid chromatography-high resolution mass spectrometry; NMR, nuclear magnetic resonance; FT-ICR-MS, Fourier-transform ion cyclotron resonance mass spectrometry; PET, positron emission tomography; VTGE, vertical tube gel electrophoresis.

highlights the need to align with the various other factors including genomic susceptibility, family history, exposures, demographic, and behavioral data. These challenges should be discussed because of future changes in the artificial intelligence-driven social environment, and the generation of new forms of elements, chemicals, and materials that may potentiate the level of complexity and noises at the cellular and molecular levels. Cancer is also a preventable disease like many other chronic conditions with better physical activity, enhanced nutritional diet, and reduced exposure to carcinogens. Association between longer intervals and later stages of diagnosis will result in reduced survival and poor quality of life [12-18].

The third challenge is crucial in the context of finding highly accurate biomarkers for the early diagnosis of cancer and importantly validation at the tissue, cells, and molecular levels by accounting for the various forms of noise that may emerge due to human physiology. Additional views including microbiome and the changing landscape of the environment should be highlighted that other than normal physiology that accounts for the noise that may deviate the accuracy of biomarkers. Among the various forms of biomarkers including circulating tumor DNA, circulating tumor cells, proteins, exosomes, and cancer metabolites, the need for the integration of data sciences and the merger of multimodal tests is pivotal in the acceleration of early diagnosis of cancer. In addition, instability in the sensitivity or specificity of the diagnostic test and misdiagnosis has become another major concern in early cancer diagnosis. Like any other area in medical practice, the grey zone also exists in pathology.

The fourth challenge is the technological advancement that will allow the creation of highly powerful molecular analytical and imaging tools to better dissect the complexity and chaos at the tissue, cell, and molecular levels. Also, the lack of sufficient knowledge and awareness among clinicians and patients regarding emerging technologies and technical terms makes communication complex in conveying results [8].

The fifth challenge concerns the assessment and implementation of potential early diagnostic tools for vulnerable cancer patients by accounting for money, time, and accessibility to the remote and poor strata of society. The absence of proper infrastructure, pathology expertise, and technologies leads to improper access to the diagnosis [8]. Altogether, this paper summarizes various key challenges in the potential uses of tumor metabolite biomarkers for the diagnosis and prognosis of cancer patients encompassing all stakeholders including patients, basic scientists, clinicians, engineers, physicists, data scientists, and AI scientists (Fig. 3).

FUTURE PERSPECTIVES ON NEW TOOLS AND APPROACHES

Given the complex nature of biological fluids and materials such as tumor tissues, serum, urine, saliva, tears, and nail clippings, achieving the desired sensitivity and specificity are formidable challenges in the use of metabolite biomarkers for the preclinical and clinical perspectives. However, approaches such as solvent-based extraction of metabolites from biological samples are routinely adopted for the purification of metabolites instead of using whole biological samples. However, this approach faces challenges in terms of the loss of crucial metabolites that are biologically available at low concentrations ranging from picomolar to micromolar. Therefore, there is a need for affordable and assisting technology that improves the existing difficulties the metabolite biomarkers discovery.

We have reported on a novel and in-house designed vertical tube gel electrophoresis (VTGE) tool that can be used as an assisting method for the purification of metabolite biomarkers from various biological fluids and materials such as tumor tissues, serum, urine, saliva, tears, and nail clippings. At the same time, VTGE-assisted purification approaches of metabolite biomarkers avoid the use of interfering solvents and the possibility of loss of low abundant metabolite biomarkers.

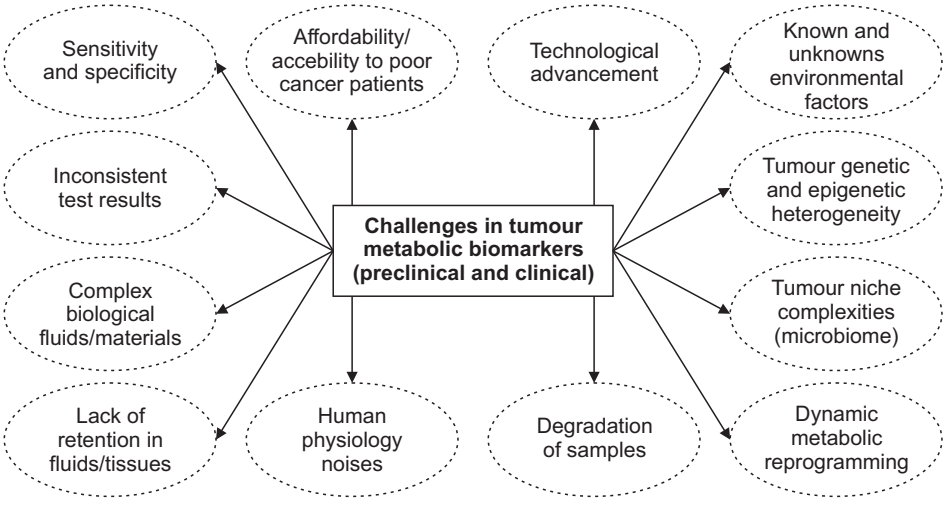

Figure 3. A summary on potential challenges that impedes the application of tumor metabolite biomarkers at preclinical and clinical levels.

Notably, the VTGE technique is uniquely designed in-house using lab plastic ware, specifically Falcon tubes of 15 and 50 mL. This innovative design expands upon the scope of Laemmli's method, which primarily utilized polyacrylamide gel for separating large biomolecules under denaturing and non-denaturing conditions. In the proposed VTGE technique, an in-house-designed VTGE tool is employed with a 15% polyacrylamide gel. This gel is standardized to allow the elution of metabolites close to or less than ~1,000 Da while trapping larger biomolecules such as proteins, DNAs, and RNAs. Importantly, the running and elution buffers used in VTGE are free of SDS, reducing agents, and other interfering reagents. This ensures that metabolites eluted from complex biological samples like urine, saliva, nails, tissues, and cells are compatible with mass spectrometry and other analytical techniques (Fig. 4) [47].

To the best of our knowledge, the utilization of polyacrylamide gel in VTGE and other customized setups, particularly beyond the scope of Laemmli's method intended for proteins and other large biomolecules, has not been previously reported. Consequently, the VTGE technique offers certain advantages over conventional protein gel electrophoresis, as well as vertical tube gel and capillary gel electrophoresis, within the context of tumor metabolite biomarkers (Fig. 5) [47-50].

In our proposed flow model, we position the in-house-designed VTGE as an assistive step in achieving sensitive and specific tumor metabolite biomarkers that is combined with feasible colorimetric assays and sophisticated mass spectrometry techniques. Therefore, we do not propose VTGE alone as a complete solution for tumor metabolite biomarkers; rather, it serves as an innovative tool to assist in the application of sophisticated mass spectrometry techniques and other analytical tools.

This is particularly important because the complex nature of biological samples and the structural similarities between expected biomarkers and large biomolecules such as proteins, RNAs, and DNAs pose significant challenges for metabolomics in cancer detection. The authors would like to contend that the VTGE tool is distinct in terms of size, idea, and concept compared to existing approaches such as capillary gel electrophoresis as conventional approaches for separating large biomolecules, particularly proteins and nucleic acids inspired by Laemmli's work in 1970 [40-43].

In brief, VTGE assisted the approach to explore metabolic biomarkers of tumor tissues, serum, urine, saliva, tears, and nail clippings by excluding macromolecules such as proteins, RNA, DNA, and other complex high-molecular weight compounds [44-50]. Therefore, the assisted approach can resolve the limitations in the early diagnosis of cancer and specifically noninvasive, accessible, and affordable approaches for the income group cancer patients. The uses of the assisted approach have been documented in the form of metabolite biomarkers such as levels of free aromatic amino acids in the nails, lipid profiles in the nails, and modified nucleosides in the urine and similar data is in progress.

In addition to the existing formidable challenges, the authors extend to propose on the relevance of metabolic biopsy (metabopsy) as a terminology. Metabopsy is coined by combining the terms "metab" from metabolic and the suffix "-opsy" derived from the Greek word "opsis" meaning medical examination or diagnosis of biological samples. Furthermore, the convergence of avenues of tumor metabolite biomarkers with artificial intelligence and machine learning models presents an encouraging avenue with significant potential. These con-

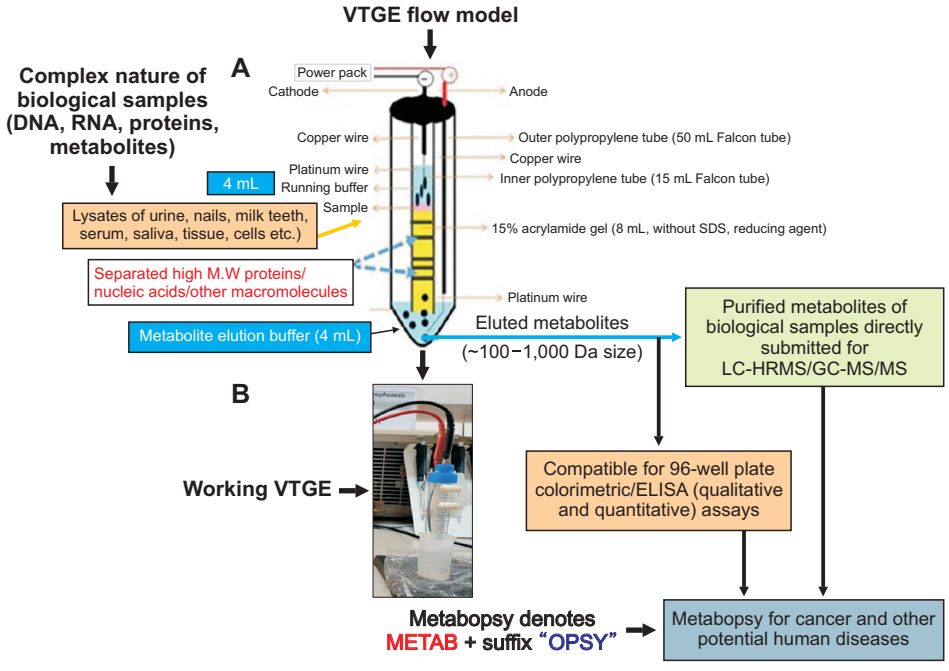

Figure 4. A flow mode of VTGE assisted metabopsy procedure of various complex nature of biological samples such as nails, urine, saliva, cells and tissues. (A) Outline model of VTGE. (B) Working model of VTGE. Purified metabolites during VTGE assisted purification is directly compatible for LC-HRMS and other analytical assays. VTGE, vertical tube gel electrophoresis; LC-HRMS, liquid chromatography-high resolution mass spectrometry; GC, gas chromatography mass spectrometry; MS, mass spectrometry.

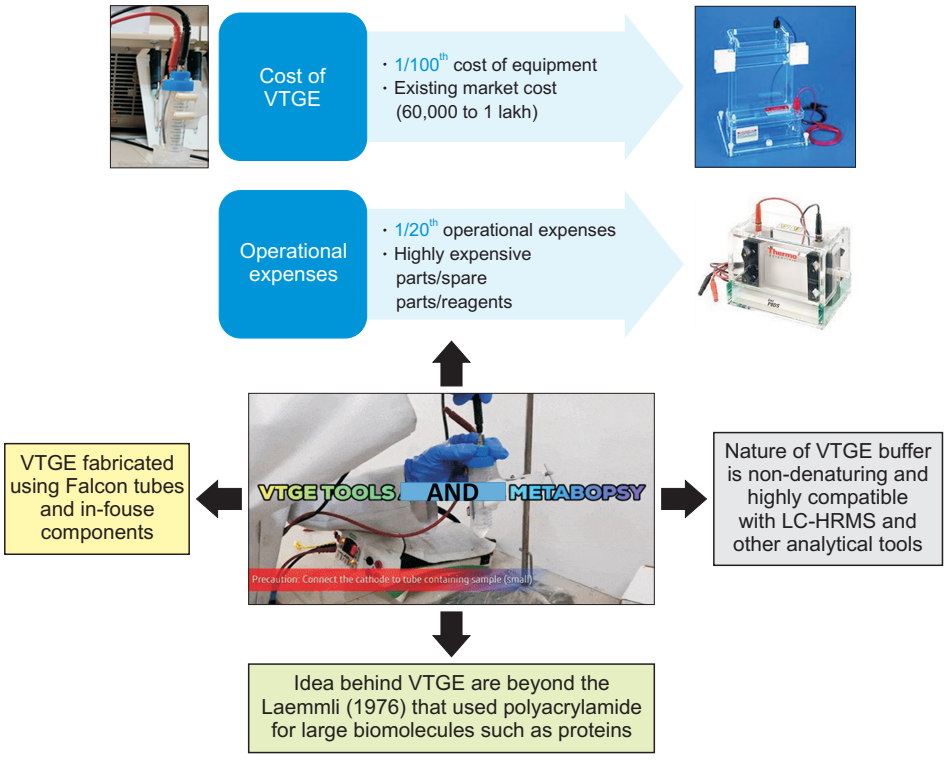

Figure 5. A summaried presentation on the scope and distinctiveness of VTGE assisted metabolote bioamrkers discovery over the existing tools and technqiues. VTGE, vertical tube gel electrophoresis; LC-HRMS, liquid chromatography-high resolution mass spectrometry.

certed strategies may offer a better prospect for early cancer diagnosis strategies.

CONCLUSION

In summary, the tumor metabolite biomarkers are proposed to be in alignment with a comprehensive framework for early cancer diagnosis. This necessitates collective deliberation amongst basic scientists and preclinical and clinical stakeholders. Preclinical and clinical experts engaged in cancer detection modalities must be engaged in collaborative and insightful discourse with molecular oncologists. This collaborative effort aims to assimilate the complexities of molecular heterogeneity, particularly at the metabolite level, as prospective reservoirs of biomarkers. Looking ahead, the potential of tumor metabolite biomarkers holds promise for expansion through the incorporation of unconventional biological substrates such as deciduous teeth, nails, tears, and sweat. Furthermore, the convergence of avenues of tumor metabolite biomarkers with artificial intelligence and machine learning models presents an encouraging avenue with significant potential. These concerted strategies may offer a better prospect for early cancer diagnosis strategies.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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